

Phenotypic Traits and Insect Catch of *Sarracenia purpurea* at Two Types of Northern
Michigan Wetlands

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Abstract

Sarracenia purpurea, commonly known as purple pitcher plants, are carnivorous plants found in wetlands with low nutrients. We compared pitcher plants between two different types of wetlands, a bog and a swale, to determine if the different habitats affected their phenotypic characteristics. We hypothesized that the pitcher plants at Grass Bay would be, on average, larger than the pitcher plants at Mud Lake Bog due to less environmental stress, and that plants with more red colored pitchers would have a higher mass of insect catch than plants with greener pitchers. Height, width, color, number of pitchers per plant, and density of insect catch were recorded at each site, and compared between the two sites. We found that Mud Lake Bog had plants with a higher average insect catch density and also higher average proportion of red pitchers to plants at Grass Bay. Our results suggest that environmental pressures do have an impact on phenotypic traits of *S. purpurea*.

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Introduction

In order to cope with poor nutrient environments, many plants have evolved alternative pathways for obtaining nutrients (Schaefer and Ruxton, 2008). One unique method that has evolved numerous times in plants is carnivory (Schaefer and Ruxton, 2008). Carnivorous plants often inhabit these low-nutrient locations, such as bogs and swales. Kettle-hole bogs were formed from retreating glaciers that left behind large blocks of ice (Dorr and Eschman 1970). Outwash materials were deposited around the large block of ice, and as it melted it left a large depression in the ground, which was then filled in by water. These lakes tend not to have drainage in or out. They accumulate *Sphagnum* moss, which causes the water to become acidic because of cation exchange during respiration. This acidity increases peat recruitment resulting in bogs with floating mats of *Sphagnum* moss (Dorr and Eschman, 1970).

Interdunal swales form from water settling in depressions between sand dune ridges along large lakes. The soils of swales are sand-based and are generally neutral or slightly alkaline due to traces of calcareous minerals (Albert, 2007). Water levels in a swale vary based on the water table of the nearby lake, and therefore swale communities consist primarily of plants that are able to survive in unstable and nutrient poor soil conditions such as rushes, sedges, and shrubs (Shumway and Banks, 2000).

One of the most prevalent types of carnivorous plants in North America is known as the pitcher plant. Pitcher plant is the common name given to hundreds of different species from predominately the *Nepenthaceae*, *Sarraceniaceae*, and *Cephalotaceae* families.

(Heywood, 1982). These plants have evolved leaves cupped in a pitcher shape, into which they lure, trap, and kill their invertebrate prey (Moran, 1996). It has been suggested that pitcher plants utilize nectar as bait, as well as visual cues in the form of ultraviolet light for luring insects to their pitchers (Schaefer and Ruxton, 2008). The inside of the pitcher plant contains a waxy layer lined with small hairs to restrict insects from escaping the pitcher (Newell and Nastase, 1998). The pitcher contains rainwater, and in some species digestive enzymes. In contrast, the purple pitcher plant, *Sarracenia purpurea*, has a variety of bacteria, protozoa, insect larva, and aquatic mites to feed on the captured invertebrates. The nitrogen from the waste of these organisms is then absorbed and used by the pitcher plant (Bradshaw and Creelman 1984).

S. purpurea is native to the Northern United States, and inhabits many of the wetlands in that region. Many studies have been done on *S. purpurea* and their efficiency in capturing prey. While Newell and Nastase (1998) have suggested that trapping efficiency in *S. purpurea* is less than 1 percent, they also proved that the benefits of catching few insects are large enough to outweigh the cost of producing the nectar used for luring. Characteristics such as coloration of pitcher, nectar content, width of pitcher, and height of pitcher have all been studied to determine what aspects create the most efficient pitcher. (Newell and Nastase, 1998, Creswell, 1998, Moran and Moran 1998, Schaefer and Ruxton 2008). The results of these previous studies are conflicting. Some studies have shown that pitchers of a red coloration have been shown to be more efficient than those of a green coloration (Newell and Nastase, 1998), but other studies have shown no correlation between pitcher color and insect catch. While (Cresswell 1998) has shown that pitcher

hood width may have a positive correlation with insect catch, Newell and Nastase (1998) did not find a significant correlation in *S. purpurea*.

Since certain traits will be advantageous in a given environment, and because pitcher plants are relatively isolated communities due to their unique habitat, we were interested in determining if pitcher plants from two locations in Cheboygan County, Michigan-Mud Lake Bog and Grass Bay-would have different phenotypic traits. Of these qualities, we were interested in how the coloration, the height, and the width of pitchers varied within and between these two sites. Would the differences between the two environments be enough to impact the phenotype of *S. purpurea*? We hypothesized that the two environments would provide sufficiently different conditions that pitcher qualities would differ between sites. Our indicator of pitcher plant success was the average density of insects that the plant was collecting per pitcher. While many studies have been done examining success of individual pitchers (Newell and Nastase, 1998, Creswell, 1998, Moran and Moran 1998, Schaefer and Ruxton 2008), we thought it would be valuable to sample the variation between all pitchers of an individual pitcher plant, and then determine the success of the entire plant, since the nutrients acquired by each pitcher benefit the entire plant. We hypothesized that, since the soil in swales is more nutrient rich and has a higher pH, pitcher plants in Grass Bay would be under less environmental stress, and therefore be less reliant on insect prey than the plants at Mud Lake Bog (Bott et al 2008). They will therefore be larger, despite not collecting a significantly greater density of insects. In addition, we hypothesized that if red coloration is indeed a better attractant for insects (Newell and Nastase 1998), then more plants in both Mud Lake Bog and Grass Bay will

have higher proportions of red pitchers, and that these redder plants will have relatively higher density of insects than greener plants.

Materials and Methods

Site Descriptions

The bog chosen to study was Mud Lake Bog located in Cheboygan County Michigan, approximately six miles northeast of The University of Michigan Biological Station. Mud Lake Bog is composed of two regions of *Sphagnum*. The more inland region of *Sphagnum* is grounded while the outer region of *Sphagnum* is floating over open water. Bog formation only occurs at one side of the lake due to wind direction. The grounded portion of the bog is characterized by tamarack, swamp laurel, cranberry shrubs, Labrador tea, sundew and pitcher plants. The species richness decreases as one moves towards open water. The pH of the bog ranges from 2.8-3.8, with the water's edge of the sphagnum being least acidic (Paranchini, unpublished).

Grass Bay, the swale of study, was located eighteen miles northeast of UMBS. The linear depressions that characterize the site as a swale run parallel to Lake Huron and alternate with dune sand deposits created by glacial activity (Rekowski). The soil consisted of sand with some accumulated organic matter. The area of the swale which contained *S. Purpurea* was also inhabited mostly by *Drosera rotundifolia*, *Drosera linearis*, *Utricularia cornuta*, *Tofieldia glutinosa*, *Triglochin martimum*, and *Rhynchospora alba*. The pH at Grass Bay ranged from 7.5-8.0 (Vande Water, unpublished).

Sampling Methods

At each site we took ten-meter transects and measured every pitcher plant within 1.5 meters on either side of the transect. At Mud Lake, we measured forty-five total plants from six transects, and at Grass Bay we measured thirty-two plants from four transects. Each pitcher plant was marked with a numbered orange flag. First we counted the number of pitchers in each plant. Then we categorized each pitcher in every plant based on color; we used an index from one to six, with one being the most green and six being the most red. In addition to categorizing them based on color, we measured the height and width of each pitcher. Heights were taken from the point where the pitcher touched the ground to the tip of the pitcher. Widths were taken at the widest section of the pitcher opening. Lastly, we extracted the water contents from each individual pitcher of our selected plants using a turkey baster or an eyedropper depending on pitcher size. Contents of all the pitchers of a single plant were placed into the same half-liter Nalgene bottle and brought to the lab for filtration. The volumes of water in each pitcher plant were obtained. In the lab, the pitcher contents were filtered using gravity filtration and a pre-weighed coffee filter. The filters were then oven-dried for 48 hours and reweighed to calculate the total weight of insects found in each plant.

Statistical Analysis

To compare characteristics between plants within a site, such as height, width and color, we performed linear regression tests. To compare a characteristic between the two sites, we ran independent samples t-tests. SPSS was used for all statistical analysis.

Results

Linear regressions comparing average height of pitchers, average widths of pitcher openings and coloration of pitchers to number of pitchers per plant showed mixed results

at our two sites. At Mud Lake Bog, the average height of pitchers was positively correlated to number of pitchers per plant ($N=45$; $p<0.001$), but at Grass Bay there was no significant relationship between height and number of pitchers (Figure 1; $N=32$; $p=0.462$). When comparing average width of pitchers in each plant to number of pitchers per plant, there was not a significant relationship at either Mud Lake Bog ($p=0.352$) or Grass Bay (Figure 2; $p=0.085$). We found that the average number of pitchers per plant differed significantly between sites, with the plants at Grass Bay ($N=32$) having an average of 13.91 (\pm SE: 1.231) pitchers per plant, and the plants at the bog ($N=45$) having an average of 5.69 (\pm SE: 0.4166) pitchers per plant (Independent samples t-test; Figure 3; $p<0.001$). The pitcher plants at Grass Bay also had, on average, significantly taller pitchers than the pitchers at Mud Lake Bog (Independent samples t-test; Figure 4; $p<0.001$). The difference in pitcher widths between the two sites approached significance with the pitchers at Grass Bay having higher average widths (Figure 5; $p=0.053$). Average pitcher volume was found to be significantly higher at Grass Bay than at Mud Lake Bog (Figure 6; $p<0.001$).

Comparing the number of pitchers per plant to average pitcher color in each plant revealed no significant relationship at either Mud Lake Bog ($p=0.727$) or at Grass Bay (Linear regression; Figure 7; $p=0.094$). We found that Mud Lake Bog had pitchers that were significantly more red than those of Grass Bay (Independent samples t-test; Figure 8; $p<0.001$).

When comparing average height of pitchers per plant to density of insect catch, there was no significant relationship at either Mud Lake Bog (Linear regression; $N=23$; $p=0.831$), or at Grass Bay (Figure 9; $N=17$; $p=0.974$). Average width of pitchers was not

related to density of insect catch (insect weight/average pitcher volume) at either the bog ($p=0.466$) or Grass Bay (Figure 10; $p=0.884$). Color of pitchers was not significantly related to weight of insect catch at Mud Lake Bog ($p=0.407$), but at Grass Bay, plants with a greater proportion of red pitchers caught a greater weight of insects (Figure 11; $p=0.022$). In contrast, comparing average pitcher color to insect density showed no significant result at Mud Lake ($p=0.9$) or at Grass Bay (Figure 12; $p=0.185$). Pitcher plants at Mud Lake Bog ($N=23$) had a significantly higher average insect density than pitcher plants at Grass Bay (Independent samples t-test; Figure 13; $N=17$; $p=0.03$).

Discussion

We found that plants at Grass Bay had, on average, pitchers that were taller and wider than the plants at Mud Lake Bog. This result is consistent with our hypothesis that larger pitchers would be found at Grass Bay. One possible explanation for this observation is that the conditions of Grass Bay foster better pitcher plant growth than the conditions of Mud Lake Bog. Another possible explanation is that pitchers at Grass Bay are harvesting greater densities of insects than the pitchers at the bog, leading to increased growth. Insect density, however, was not found to be higher at Grass Bay. Finding larger pitchers, but lower insect catch density, at Grass Bay is consistent with the Newell and Nastase (1998) finding that pitcher growth is not dependent only on insect catch. Had we found a higher average insect density at Grass Bay, the study done by Cresswell (1998) would have been supported. Our results actually showed a higher average insect density at Mud Lake Bog, despite the smaller size of the pitchers. This could possibly be attributed to effects of pitcher color. If Grass Bay is indeed more nutrient rich, then it is possible that pitchers there can rely more on the environment rather than on insect catch for nitrogen. Green

pitchers have greater photosynthetic potential than red pitchers, due to differential absorption of light (Bott et. al., 2008). Red pitchers have been shown to attract insects better than green pitchers due to reflection of ultraviolet light (Cresswell, 1996). Therefore, plants at the bog, the more nutrient-poor environment, would benefit from having more red pitchers to attract insects, whereas plants at Grass Bay would benefit by having greener pitchers, increasing their photosynthetic potential. This explanation of pitchers being red to catch insects in nutrient-poor environments is supported by our finding of redder plants at Mud Lake Bog. Another possible relationship between color and density could be that if some pitchers are red in color, regardless of environmental conditions, they will naturally be less photosynthetic than green pitchers, and will therefore be required to catch more insects to support growth. This relationship is not supported by our results because we found a significant difference in average color between our two sites, indicating that the environment does affect pitcher color.

Although we found more red pitchers and higher insect densities at Mud Lake Bog than at Grass Bay, our second hypothesis that plants with a higher proportion of red pitchers would catch a higher density of insects is not supported because we found no direct relationship between color and insect density in plants at either site. This result contrasts with studies that have found correlation between red pitcher color and higher insect catch (Newell and Nastase, 1998). We also hypothesized that different conditions at the two sites would result in differences in phenotypic characteristics. In addition to height differences between sites, we found that plants at Grass Bay had, on average, more pitchers than plants at Mud Lake Bog. This could be explained by the environment being more stressful at Mud Lake Bog (Bott et. al., 2008), potentially limiting the number of pitchers

that a plant has the resources to produce. It is also possible that having more pitchers is beneficial to plants at Grass Bay because the plant as a whole receives the photosynthetic benefits of each pitcher.

Future studies should include more pitcher plants at each site. Some of our results may not have had significant correlations because of a small sample size. Another study could compare multiple bogs to multiple swales, therefore increasing sample size and eliminating microsite variation. The pH values at Mud Lake Bog increase moving out toward open water, possibly affecting stress levels of pitcher plants, which we did not control for in our study. The study could also compare plant phenotypes between different sites of the same type. Future experiments could also benefit from more nutrient sampling at each site, as a point of comparison between sites and as a measure of variability within sites. Had we measured nitrogen content of the environment surrounding each pitcher plant, we could have made more direct comparisons of phenotype and insect catch density to environmental conditions.

Our approach to this topic was a unique way of addressing pitcher plant research. Other studies have focused more on the characteristics of individual pitchers; our study took a more holistic approach and focused on the entire plant, which represents the unit of selection. We found that that coloration and insect catch density of entire plants varied between Grass Bay and Mud Lake Bog, suggesting that environmental factors do impact phenotypic attributes of pitchers. We believe that this approach should be used in the future for studying adaptations of pitcher plants to low-nutrient environments.

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We would like to acknowledge The University of Michigan Biological Station for providing us with the materials necessary to conduct this study. We would also like to acknowledge Jordan Price for assisting us with statistical analysis and providing us with valuable insight throughout our experiment. We would also like to thank Jenn Rowe for words of encouragement and for help with project design.

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Appendix

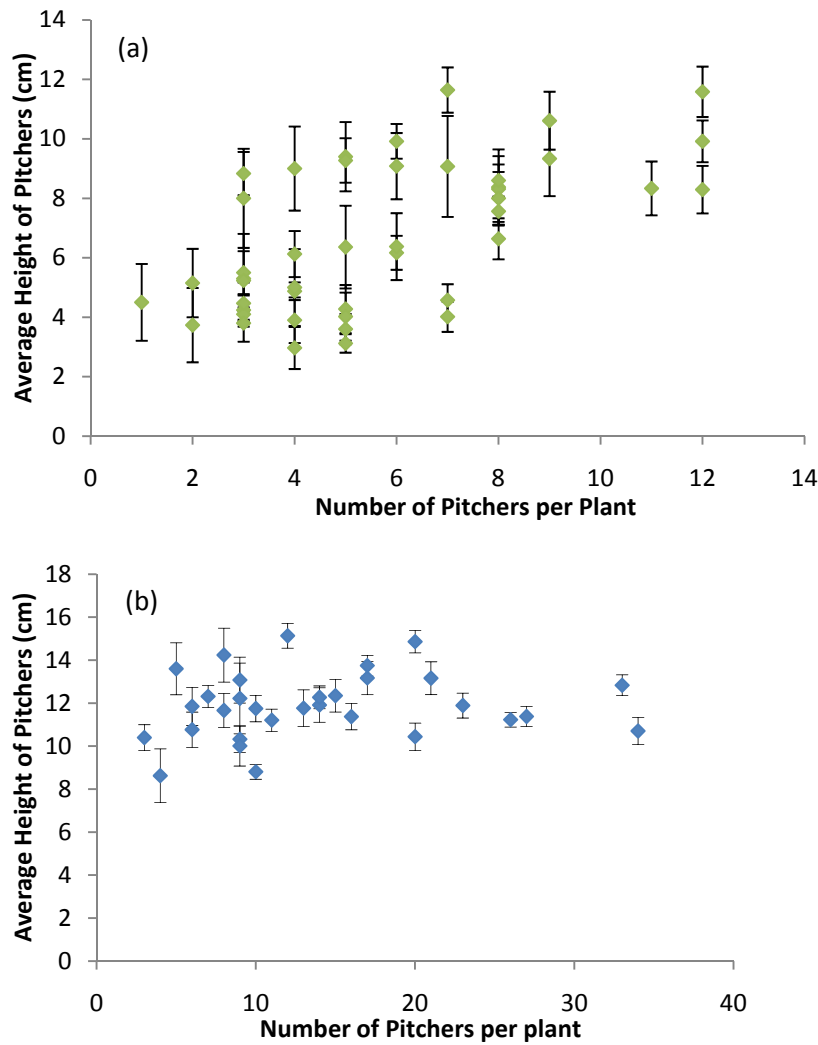


Figure 1. Average height of pitchers (\pm SE) in each plant at (a) Mud Lake Bog increased significantly with increasing number of pitchers per plant (linear regression; $R^2=0.549$; $N=45$; $p<0.001$), whereas average height of pitchers (\pm SE) in plants at (b) Grass Bay showed no significant relationship to number of pitchers per plant (linear regression; $R=0.172$; $N=32$; $p=.462$).

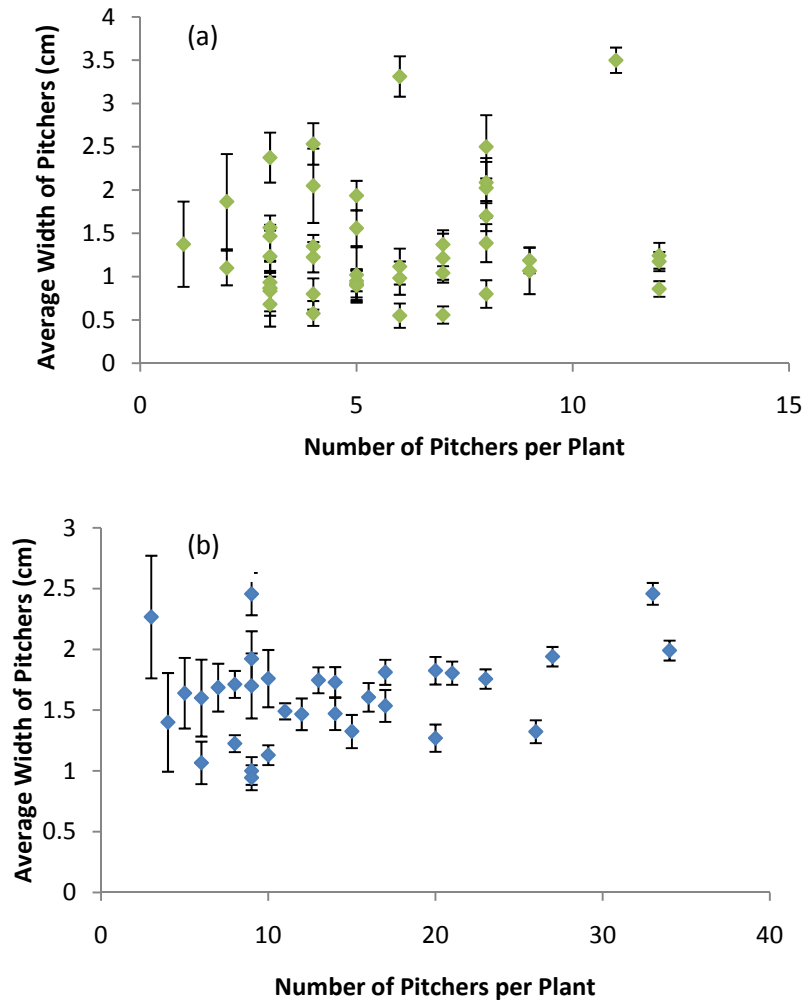


Figure 2. Average width of pitchers (\pm SE) from plants at (a) Mud Lake Bog and (b) Grass Bay were not significantly related to the number of pitchers per plant. Mud Lake: (Linear regression; $R^2=0.142$; $N=45$; $p=0.352$). Grass Bay: (Linear regression; $R^2=0.309$; $N=32$; $p=0.850$)

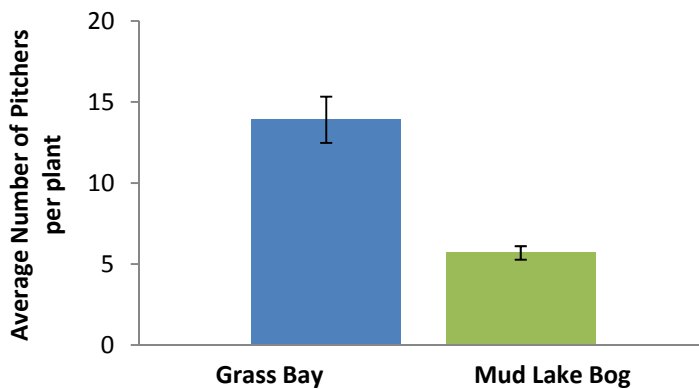


Figure 3. On average, plants at Grass Bay (\pm SE: 1.232113), (N=32) had significantly more pitchers than plants at Mud Lake Bog (\pm SE: 0.416603) (N=45; Independent samples t-test; $p<0.001$).

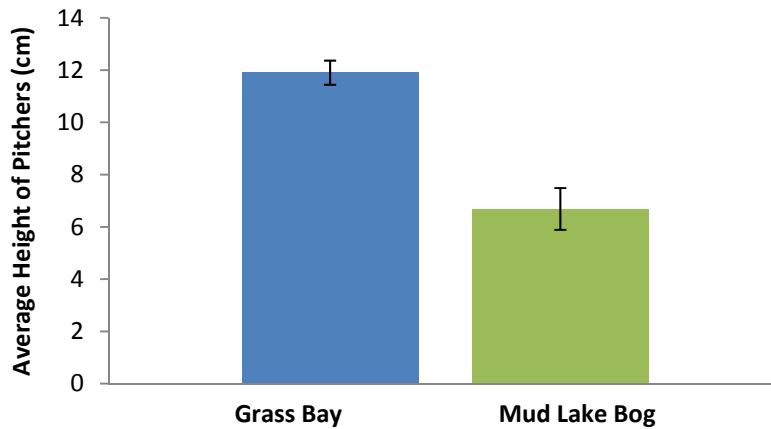


Figure 4. Pitchers from plants at Grass Bay (\pm SE: 0.273956) (N=32) were significantly taller compared to pitchers from plants at Mud Lake Bog (\pm SE: 0.368228) (N=45; Independent samples t-test; $p<0.001$).

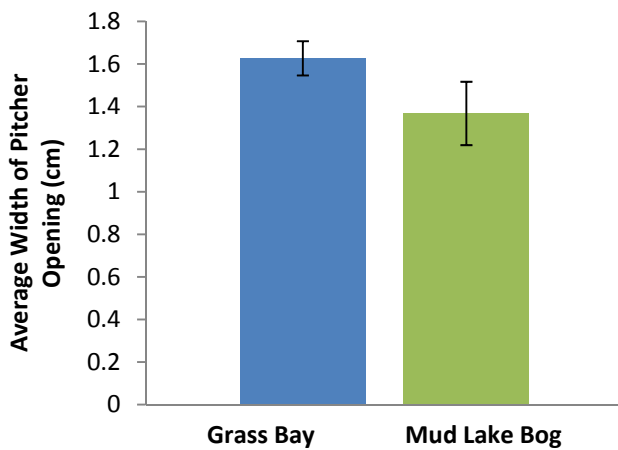


Figure 5. Average widths of pitchers from plants at Mud Lake Bog (\pm SE: 0.066178) (N=45) showed a moderately significant difference to average widths of pitchers from plants at Grass Bay (\pm SE: 0.100594) (N=32; Independent samples t-test; $p=0.053$), with the pitchers at the bog being wider than those at Grass Bay.

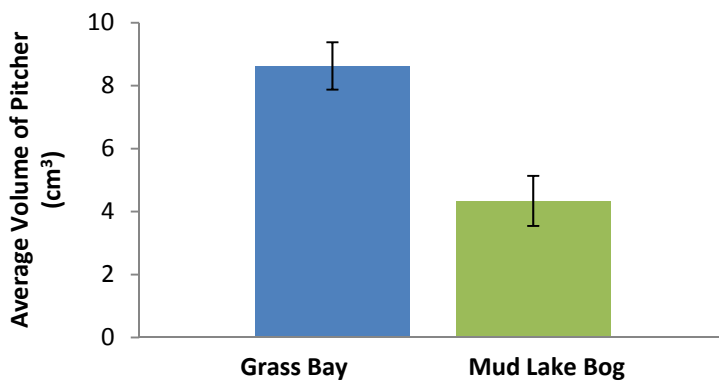


Figure 6. Pitchers at Grass Bay (\pm SE: 0.721545) (N=32) had, on average, larger volumes than pitchers at Mud Lake Bog (\pm SE: 0.795414) (N=45; Independent samples t-test; $p<0.001$).

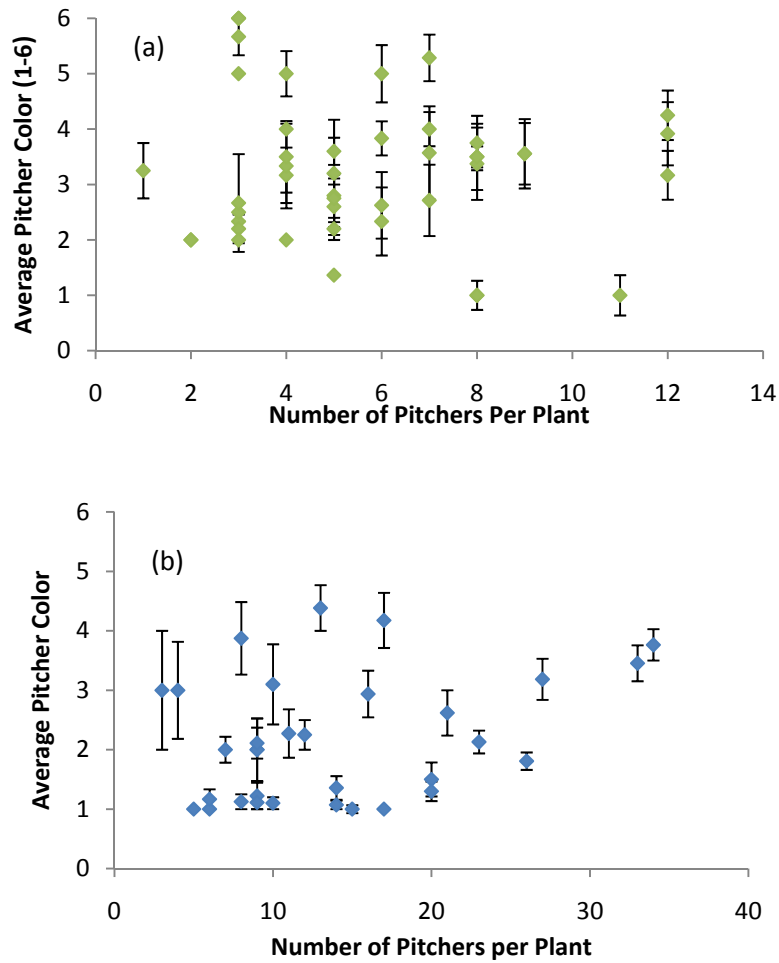


Figure 7. Average pitcher color (\pm SE) of plants at (a) Mud Lake Bog (Linear regression; $R^2=0.003$; $N=45$; $p=0.727$) and (b) Grass Bay (Linear regression; $R^2=0.301$; $N=32$; $p=0.094$) showed no significant relationship to the number of pitchers per plant.

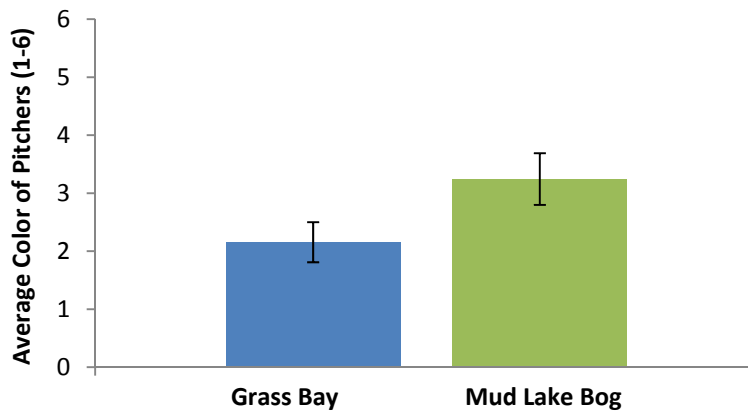


Figure 8. The average color scale of pitchers across plants at Mud Lake Bog (\pm SE: 0.185517) (N=45) was significantly higher (more red) than the color of pitchers at Grass Bay (\pm SE: 0.187261) (Independent samples t-test; N=32; $p<0.001$).

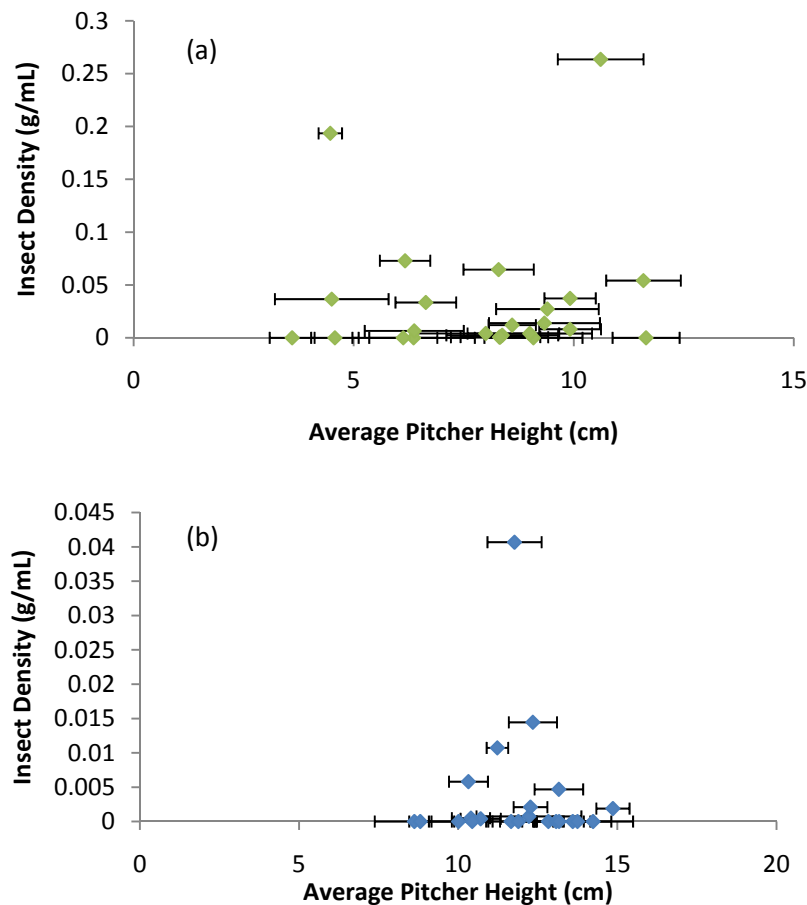


Figure 9. Density of insects caught by each pitcher plant at (a) Mud Lake Bog (Linear regression; $R^2=0.002$; N=23; $p=0.831$) compared to average pitcher height (\pm SE) showed no significant relationship. Similarly, density of insects caught by each pitcher plant at (b) Grass Bay (Linear regression; $R^2<0.001$; N=17; $p=0.974$) showed no significant relationship to average height of pitchers (\pm SE).

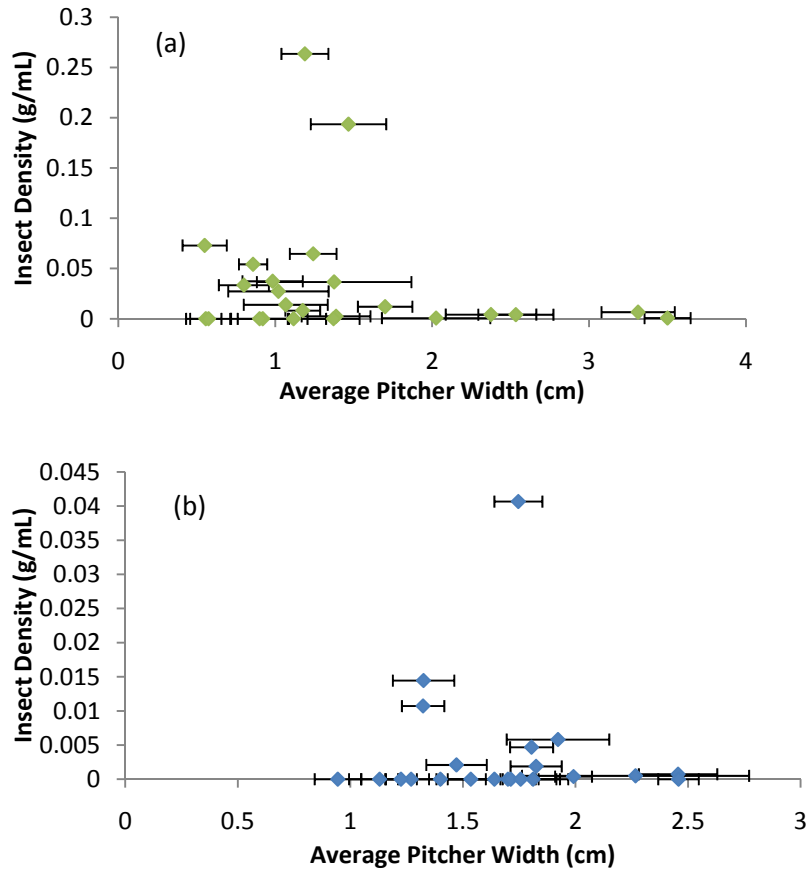
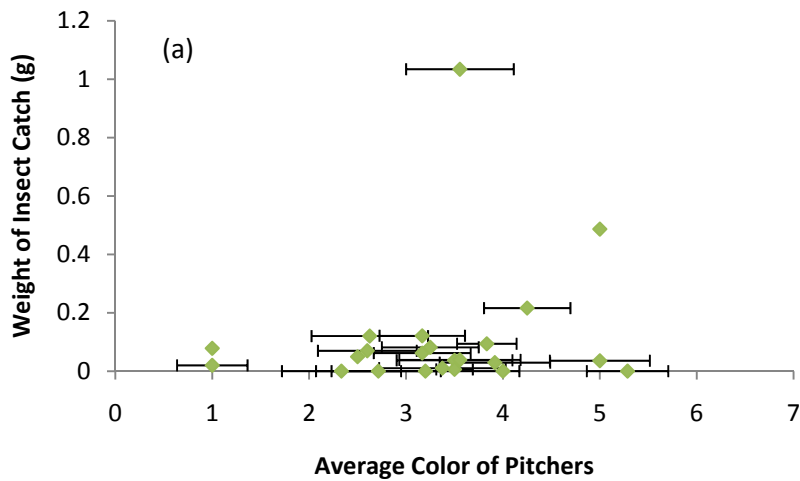


Figure 10. Density of insect catch by pitcher plants not significantly related to average width of pitcher opening (\pm SE) at (a) Mud Lake Bog (Linear regression; $R^2=0.024$; $N=23$; $p=0.466$) or (b) Grass Bay (linear regression; $R^2=0.001$; $N=17$; $p=0.884$).



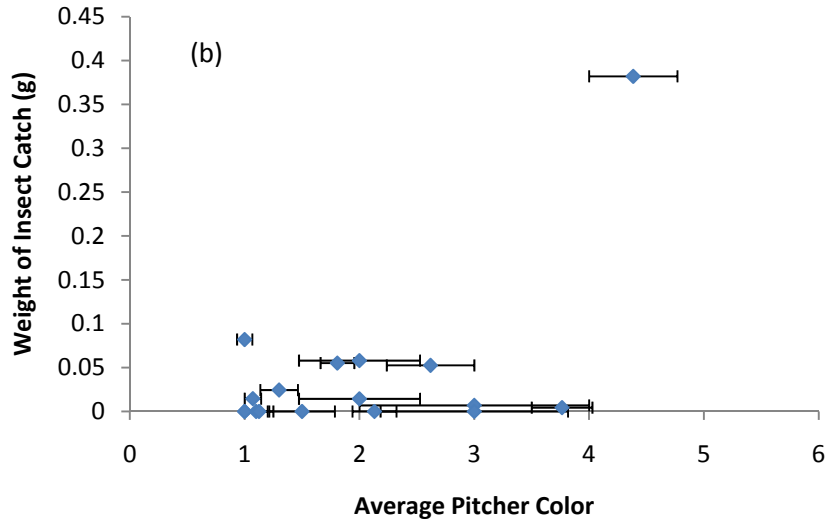
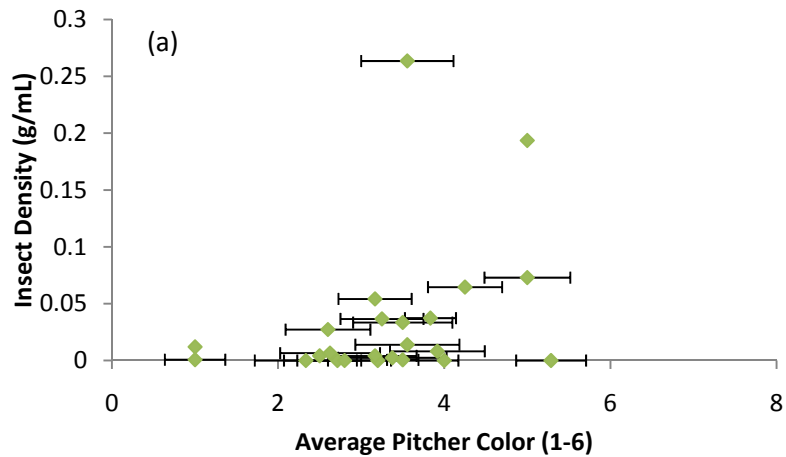


Figure 11. Weight of insect catch was not significantly related to average pitcher color (\pm SE) at (a) Mud Lake Bog (Linear Regression; $N=23$; $p=0.401$), but at (b) Grass Bay, there was a significant positive correlation between color and weight of insect catch (Linear regression; $N=17$; $p=0.022$)



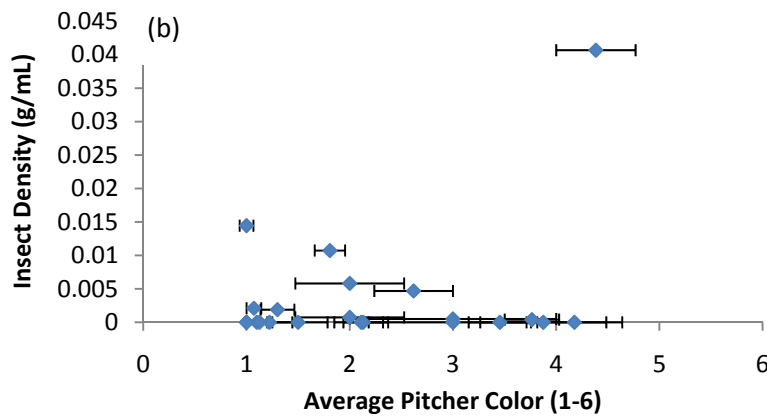


Figure 12. Density of insect catch by pitcher plants at (a) Mud Lake Bog showed no significant relationship to average color of pitchers (\pm SE) (linear regression; $R^2=0.125$; $N=23$; $p=0.90$). Similarly, density of insect catch by pitcher plants at (b) Grass Bay was not significantly related to average pitcher color (\pm SE) of each plant (linear regression; $R^2=0.086$; $N=17$; $p=0.185$).

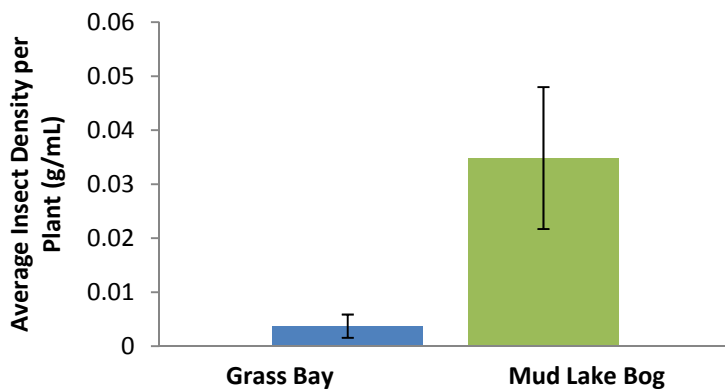


Figure 13. The average insect density per pitcher at Mud Lake Bog (\pm SE: 0.01314) ($N=23$) was significantly higher than the density per pitcher at Grass Bay (\pm SE: 0.001641), ($N=17$; Independent samples t-test; $p=0.030$).